A novel genetic region flanks the plasmid-encoded blaNDM-1 isolated from a patient in Rhode Island in 2012

Louis B. Rice¹,²
Amelia Tait-Kamradt¹

From the Department of Medicine, Rhode Island Hospital¹ and the Warren Alpert Medical School of Brown University², Providence, RI

Corresponding Author:
Louis B. Rice. M.D.
Department of Medicine
Rhode Island Hospital
593 Eddy Street
Providence, RI 02906
Telephone: 401-444-5678
Fax: 401-444-5492
e-mail: lrice@lifespan.org
As of February of this year, 24 cases of NDM-producing Enterobacteriaceae have been reported in the United States(1). The majority of these reports involved one or two isolates, except the most recent, in which eight patients were reportedly infected or colonized(1).

In June, 2012, two NDM-1 producing *Klebsiella pneumoniae* strains were reported from patients in Rhode Island, one of whom had returned after a hospitalization in Vietnam(2). We now report the molecular details of the *bla*<sup>NDM-1</sup> region of one of the plasmids (from the index case – pRI1) encoding this enzyme and compare it with previously reported NDM plasmids.

Hybridization studies indicated that the *bla*<sup>NDM-1</sup> gene was located on a >200 kb plasmid that was not transferable to *Escherichia coli* J53rif<sup>r</sup> in filter matings. Total *K. pneumoniae* DNA was used to transform *E. coli* DH10B by electroporation, selecting on BHI agar plates with ampicillin (25 µg/ml). The presence of *bla*<sup>NDM-1</sup> in the transformant was confirmed by PCR using custom primers (ndmF: 5'-gaaactgtcgcacctcatgtttg-3'; ndmR – 5'-gcccagcttcgcataaaacg – 3'). An 9.3 kb *bla*<sup>NDM-1</sup>-hybridizing HindIII fragment was ligated to HindIII-digested pACYC184 and introduced into *E. coli* DH10B, resulting in pRIH26. The HindIII fragment conferred resistance to meropenem, gentamicin, and amikacin.

The complete HindIII fragment was sequenced by primer walking using capillary Sanger sequencing (GeneWiz, South Plainfield, N.J.); the sequence has been deposited in GenBank under accession no. KC999038. Assembly and alignments were carried out using DNASTar Lasergene Core suite (DNASTar, Inc., Madison, Wi). Sequences were identified by nucleotide BLAST. The results are shown in the Figure. The coding sequence of *bla*<sup>NDM-1</sup> and the sequences downstream of *bla*<sup>NDM-1</sup> extending to the HindIII cloning site were identical to sequences found on pNDM-HK(3), originally isolated from a patient in Hong Kong, and pNDM-OM(4), isolated from a patient in the Sultanate of Oman. This region contains the *bla*<sup>NDM-1</sup> ORF, the *trpF* ORF (in the same orientation) and the structural and regulatory genes of...
blaDHA-1. In contrast, the region upstream of blaNDM-1 differed completely from pNDM-HK and pNDM-OM and from any of the NDM regions on plasmids currently reported. The upstream HindIII site interrupts the 5’ end of the qnrB38 gene, such that the cloned HindIII fragment does not confer reduced susceptibility to fluoroquinolones. Other identifiable regions upstream of blaNDM-1 include an intact copy of IS3000, an rmtC gene (conferring the aminoglycoside resistance) and two open reading frames comprising a complete copy of IS1. Separating qnrB and IS3000 is a short, intragenic region from ISCR rmtC, whereas between rmtC and IS1 lies a region homologous to the right end of IS youtube.

The data reported herein suggest that pRI1 derived from the IncI/M plasmids pNDM1-HK and pNDM1-OM, since the region downstream of blaNDM-1 was identical to these two plasmids. The differences in the upstream region appear to involve rearrangements due to the activity of one or several IS elements, in this case placing genes for broad-spectrum β–lactam, fluoroquinolone, and aminoglycoside resistance in close proximity. Attempts to characterize the incompatibility group of this plasmid by PCR amplification(s) were unsuccessful, implying recombination of this blaNDM-1 region with a plasmid of a different incompatibility group. The data presented in this letter reinforces that genetic flexibility and geographic mobility of this important antimicrobial resistance genotype.

References


Figure: Comparison of open reading frame map from pRIH26 (9.3 kb HindIII fragment from pRIH ligated to pACYC184) and previously described NDM-1-encoding plasmids pNDM-HK and pNDM-OM. The regions extending downstream of the \textit{bla}_{NDM-1} genes are identical in all three plasmids. The regions upstream of the \textit{bla}_{NDM-1} are identical in pNDM-HK and pNDM-OM and differ significantly from the region upstream of \textit{bla}_{NDM-1} in pRIH26. The open reading frames are detailed below each map.